

# DETERMINING CRITICAL N LOADS TO SUBALPINE LAKES IN THE PACIFIC NORTHWEST

*Final Report- September 2009*

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## INTRODUCTION

Long-term research in Rocky Mountain National Park (ROMO) demonstrated that increased inputs of atmospheric nitrogen changed the water quality of high-elevation lakes and shifted their trophic states. Identifying the level at which this shift occurred gave the National Park Service (NPS) the ability to identify a “critical load” for eutrophication effects on high alpine lakes at ROMO. Identifying the critical load enabled the NPS to work cooperatively with the EPA and state regulatory agencies to conduct further research and reduce emissions. This model is now being adopted across additional high-elevation systems in the West, including the Pacific Northwest (PNW). Concerns over atmospheric deposition in the PNW for high-elevation lake ecosystems include both eutrophication and acidification effects.

The critical load for alpine lake ecosystems in ROMO is based on the response of algae to lake water chemistry. Algae are used in these types of studies because they are often the first organisms to respond to environmental changes, providing some of the earliest warnings of ecosystem change.

In this study, we focus on identifying the response of algae in two PNW lakes on federal lands to the nutrient enrichment effect of enhanced N deposition, as this would occur at lower deposition rates than acidification effects. To assess the response of phytoplankton community structure to different levels of nitrogen (N) deposition, we conducted a set of cubitainer experiments along an established N gradient in two different lakes in Washington state, Dorothy and Cora. Dorothy is located in the Alpine Lakes Wilderness, while Cora is situated in the Gifford Pinchot National Forest.

## METHODS

### ***Lake surveys***

Vertical profiles of a suite of physical, chemical, and biological parameters were collected for each lake on the day that each experiment was established. A Hydrolab multiparameter probe was used to assess temperature and conductivity throughout each water column. Water samples for chlorophyll and nutrient measurements were collected using a van Dorn horizontal bottle. Chlorophyll *a* was measured by immediately filtering 0.5 L of lake water through a Whatman GF/F filter. Filters were placed in petri dishes, wrapped in foil, and stored in a freezer until analysis. All samples were analyzed within one week of collection. Chlorophyll was extracted in 90% acetone; the extract was clarified by centrifugation. Chlorophyll *a* concentrations were determined fluorometrically, as described in APHA et al. (2000). Dissolved nutrient samples were filtered through pre-rinsed 0.4- $\mu$ m polycarbonate filters and analyzed by standard methods (APHA et al., 2000). Nitrate plus nitrite was measured by the cadmium

reduction method, and SRP by the ascorbic acid method. Dissolved Si was measured by the heteropoly blue method. Material retained on the polycarbonate filters was analyzed for particulate P by persulfate digestion followed by measurement of SRP. Particulate C and N were collected on 0.70  $\mu\text{m}$  Whatman GF/F filters and measured via combustion and gas chromatography with a Costech elemental analyzer. Particulate nutrient values were used to calculate seston C:N:P ratios.

### ***Experiments***

Experiments were conducted during July 20-30, 2008. Lake water with natural phytoplankton assemblages was collected from the metalimnion of each lake and added to a series of 1-L cubitainers. Water was pre-filtered through 212  $\mu\text{m}$  mesh to remove large zooplankton grazers. Five N treatments were established in triplicate along an N gradient ranging from slight to high N enrichment: 0.05, 0.1, 0.5, 1.0, and 8.0  $\mu\text{M}$  N as nitrate. Nitrogen was added in the form of  $\text{NaNO}_3$ , as in past enrichment experiments (Saros et al. 2005a). A single P enrichment treatment of 1.0  $\mu\text{M}$  P as phosphate, in excess of typical algal requirements, was also established in triplicate to assess P limitation. In addition, a control with no nutrient additions was established in triplicate as well, resulting in 7 treatments x 3 replicates = 21 bottles per lake.

Cubitainers were clipped with fishing line and caribeeners to a plastic rack suspended at 0.5 m in the water column. The rack was anchored at a suitable depth, and put in place via an inflatable raft. The experiment lasted for 8 days. Initial (day 1) chlorophyll and nutrient samples were collected from each lake. At the end of the experiment, phytoplankton and chlorophyll samples were collected from each cubitainer to assess algal community composition and total algal production (i.e., chlorophyll concentration). The cubitainers were backpacked out before sample removal from them, and the unused water in them after sample collection was disposed of outside of the wilderness areas.

One-way ANOVAs were conducted to determine significant effects ( $p \leq 0.05$ ) of the nutrient treatments.

### ***Sediment cores***

To assess whether changes in water chemistry have already occurred as a consequence of enhanced nitrogen deposition to the study lakes in Washington, we examined sedimentary diatom assemblages from the last 100 years in both lakes. Short sediment cores, collected with a gravity corer, were sectioned into 0.5 cm increments. The chronology of sediments was based on  $^{210}\text{Pb}$  distillation and alpha spectrometry methods (modified from Eakins and Morrison, 1978), and dates determined according to the constant flux:constant sedimentation rate (CF:CSR) model (Oldfield and Appleby, 1984).

Diatom slides were prepared by digesting sediment samples with 30%  $\text{H}_2\text{O}_2$ . The processed samples were settled onto coverslips and mounted onto slides with Naphrax<sup>®</sup>. A minimum of 300 valves per slide were counted under oil immersion on an Olympus BX-51 microscope with differential interference contrast under oil-immersion at 600X magnification. Diatom taxonomy was based primarily on Krammer and Lange-Bertalot (1986-1991) and Camburn and Charles (2000).

Environmental inferences from the diatom records are based on the diatom calibration set of Eilers et al. (1998). Total phosphorus (TP) and pH were inferred for each sediment core by calculating values with the weighted averaging equation:

$$x_i = \frac{\sum_{k=1}^m y_{ik} \hat{u}_k}{\sum_{k=1}^m y_{ik}}$$

where:

$x_i$  is the estimate of environmental variable  
for fossil sample  $i$

$y_{ik}$  is the abundance of taxon  $k$  in fossil sample  $i$

$\hat{u}_k$  is the optimum of species  $k$

## RESULTS & DISCUSSION

Initial conditions in both lakes reveal relatively low productivity lakes, as indicated by low chlorophyll concentrations (Table 1). In comparison to subalpine lakes of the central Rocky Mountains (Saros et al. 2005b), nitrate concentrations in Dorothy were moderate, and high in Cora. Both lakes had undetectable phosphate levels (< 1 ug/L), but high silica.

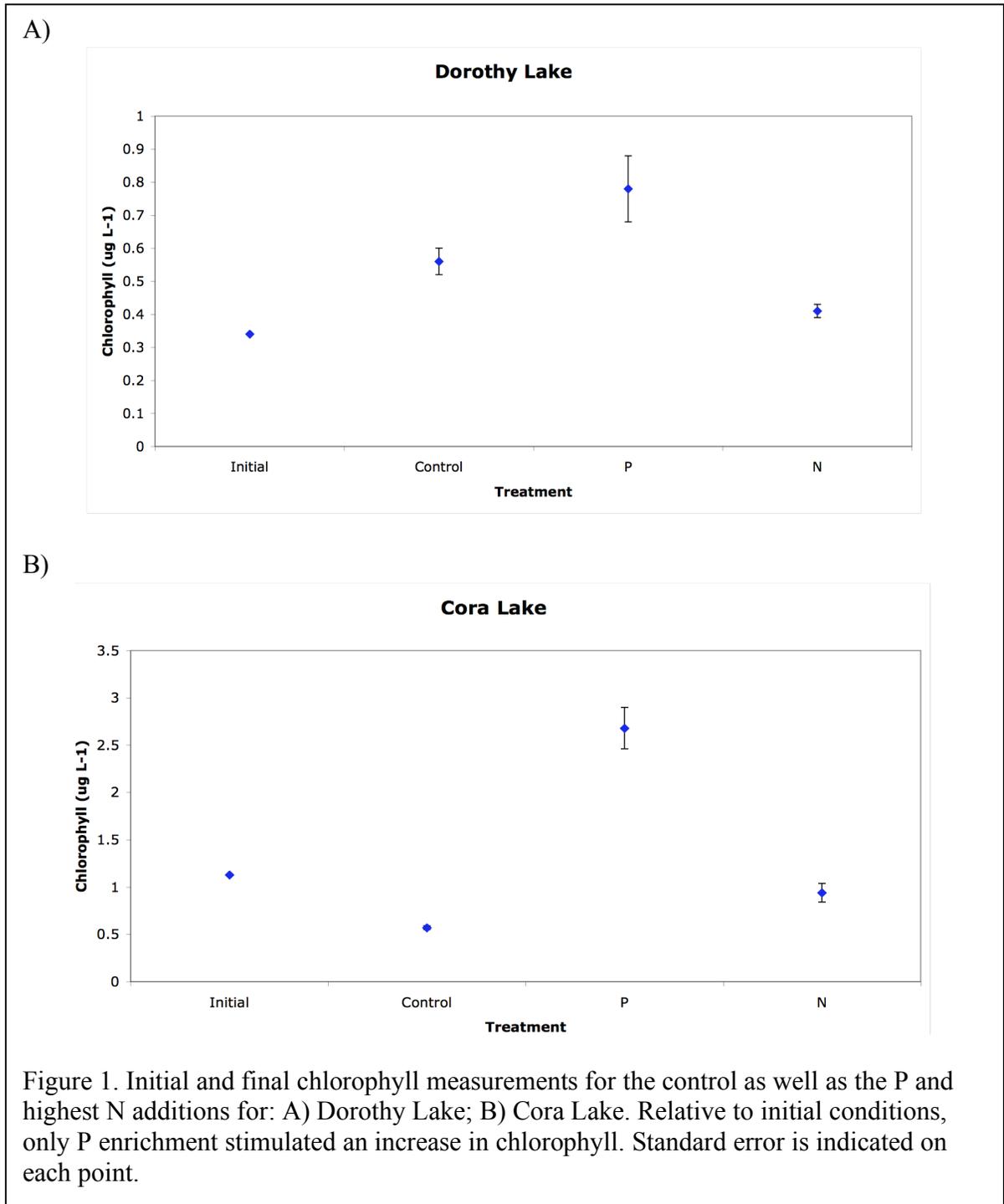
**Table 1. Initial chlorophyll, dissolved nutrient concentrations, and seston nutrient ratios in both experimental lakes.**

<u>Lake</u>	<u>Chl</u> (ug/L)	<u>Nitrate</u> (ug/L)	<u>Phosphate</u> (ug/L)	<u>Silica</u> (mg/L)	<u>C:N</u>	<u>C:P</u>	<u>N:P</u>
Dorothy	0.34	11	< 1	1.55	10.9	454	41.5
Cora	1.14	71	< 1	6.31	10.9	313	28.6

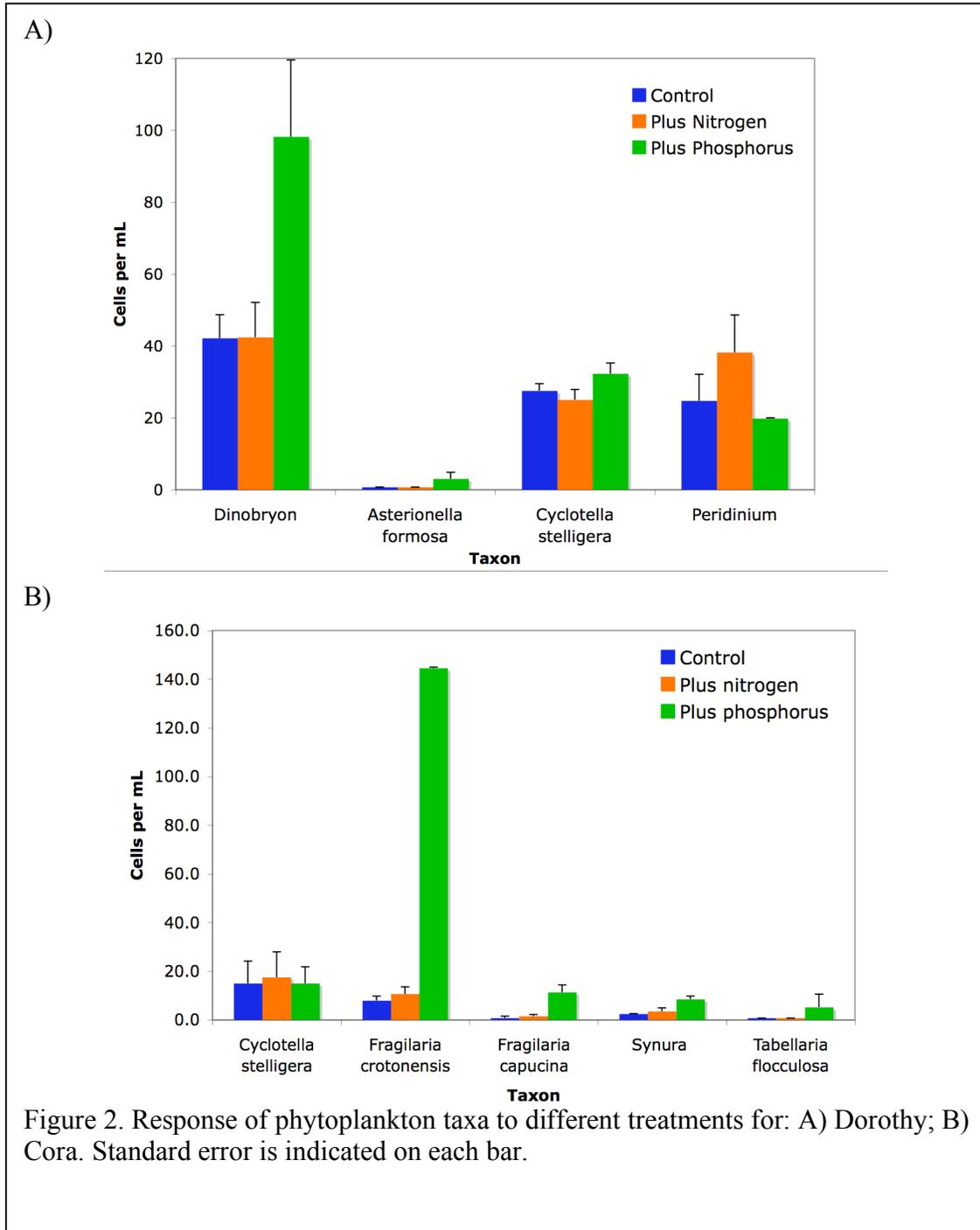
Seston ratios suggest P limitation in both lakes (Table 1), as C:P ratios are higher than 258 and N:P ratios are greater than 23 (Wetzel, 2001).

Chlorophyll analyses from the experimental treatments revealed a positive response only to P enrichments in both lakes (Fig. 1; Dorothy:  $p=0.03$ ; Cora:  $p<0.001$ ), indicating

strong P limitation in both systems. In contrast, chlorophyll increases were not stimulated in any of the N treatments (data only shown for highest N level in Fig. 1), suggesting no N limitation in these lakes. These results are consistent with the seston data from each lake, which also indicated P limitation. The strong limitation by P in these two lakes suggests that N enrichment effects will not occur in these lakes.

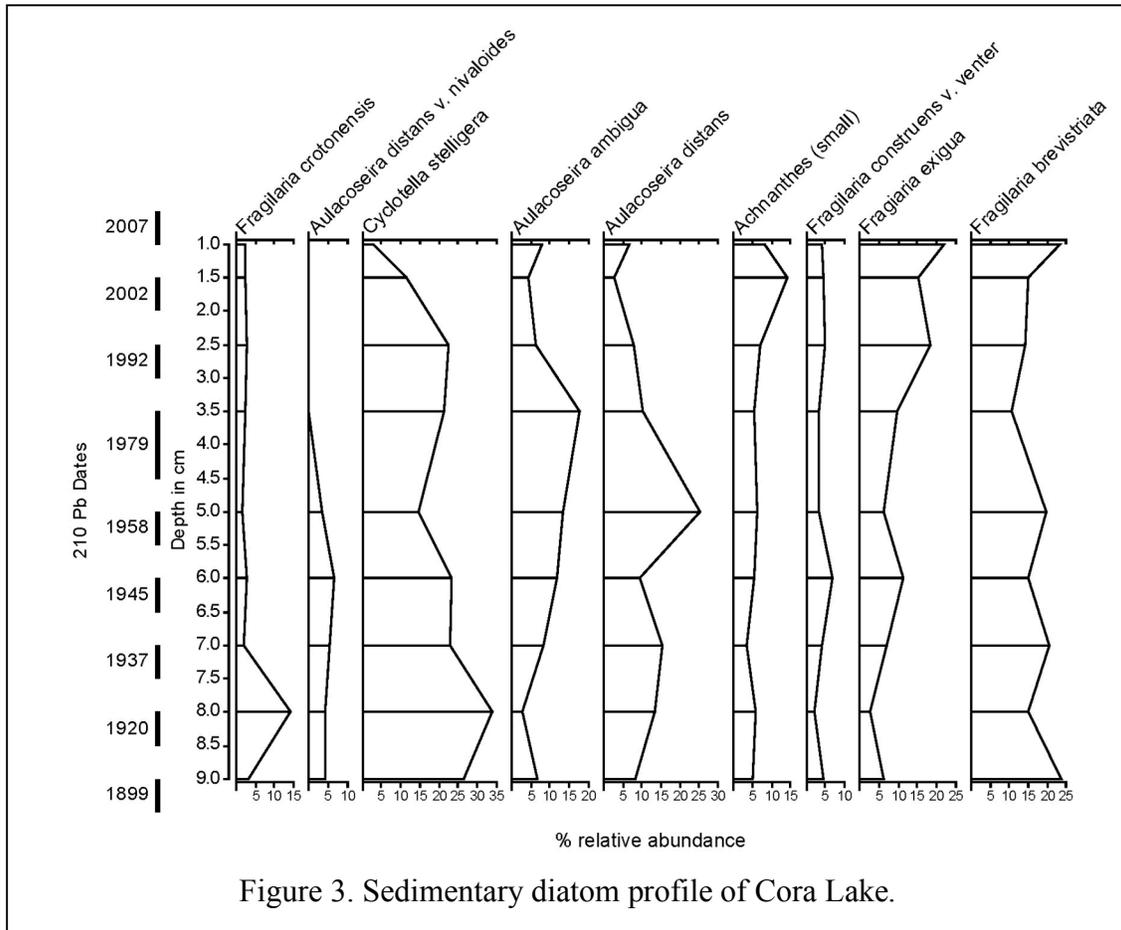


Analyses of phytoplankton community structure further support strong responses to P, not N, enrichment. In Dorothy Lake, *Dinobryon*, a genus of chrysophyte algae common in low conductivity, low nutrient lakes, responded to P enrichment ( $p=0.047$ ). In Cora Lake, the abundance of the diatom, *Fragilaria crotonensis*, increased dramatically in response to P enrichment ( $p<0.001$ ). In many freshwaters, this species is an indicator of moderate P enrichment (i.e., mesotrophic conditions). In alpine lakes, it can be an



indicator of N enrichment, but once N limitation is alleviated, this species often shows a strong response to P enrichment (Saros et al. 2005a). The diatoms *Fragilaria capucina* ( $p=0.05$ ) and the synurophyte *Synura* ( $p=0.05$ ) also increased with P enrichment in this lake.

The sedimentary diatom profile from Cora Lake reveals that changes in diatom taxa have occurred over the last century (Fig. 3). *Fragilaria crotonensis*, a species that responded to P in the experiments, was more abundant early in the 20<sup>th</sup> century compared to today. *Cyclotella stelligera* has also generally declined over the last century, particularly in the last 15 years. Small *Achnanthes* species, *Fragilaria exigua*, and *Fragilaria brevistriata* are the most abundant taxa in recent sediments. While the abundances of these diatom taxa have fluctuated over the last century, diatom-inferred pH values changed very little (Fig. 4), ranging only between 6.76 to 7.17. Diatom-inferred TP also showed only small changes (Fig. 5), ranging between 10.46 to 11.74. These diatom-inferred values suggest essentially no change in these two water chemistry parameters during the last century.



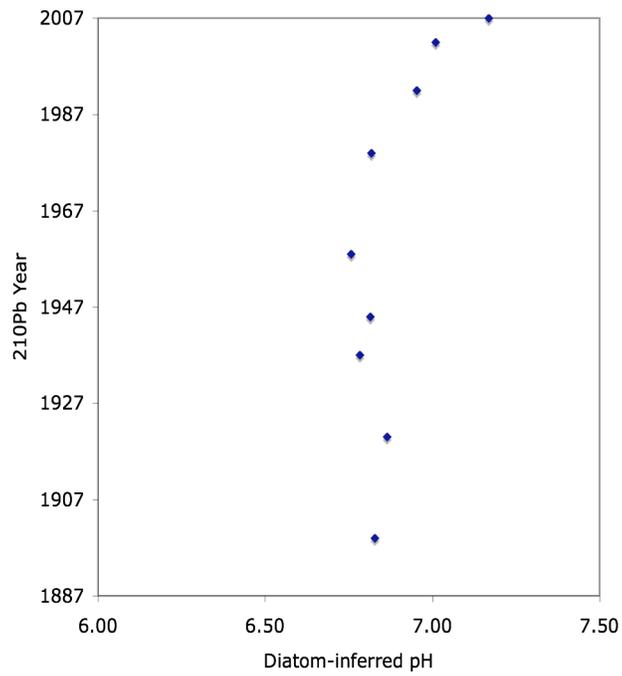


Figure 4. Diatom-inferred pH for Cora Lake.

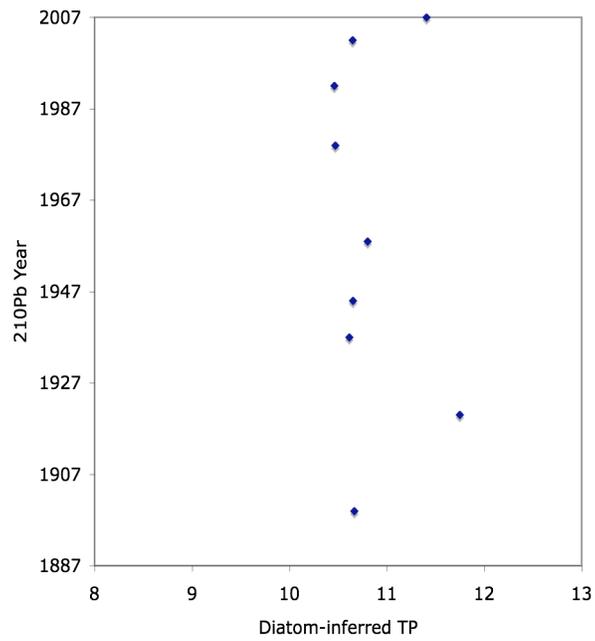


Figure 5. Diatom-inferred total phosphorus (TP) for Cora Lake.

The sedimentary diatom profile from Dorothy Lake reveals relatively few changes in the abundances of various diatom taxa over the last century (Fig. 6). The greatest change is the drop in the relative abundance of *Cyclotella stelligera* early in the 20<sup>th</sup> century. Small *Achnanthes* species also fluctuated over the last 100 years, varying from about 15 to 30% of the total assemblage. Diatom-inferred pH values were essentially unchanged (Fig. 7), ranging only between 6.34 to 6.43. Diatom-inferred TP was also essentially unchanged (Fig. 8), ranging between 7.78 to 8.68. Again, as with Cora Lake, these diatom-inferred values suggest essentially no change in these two water chemistry parameters during the last century.

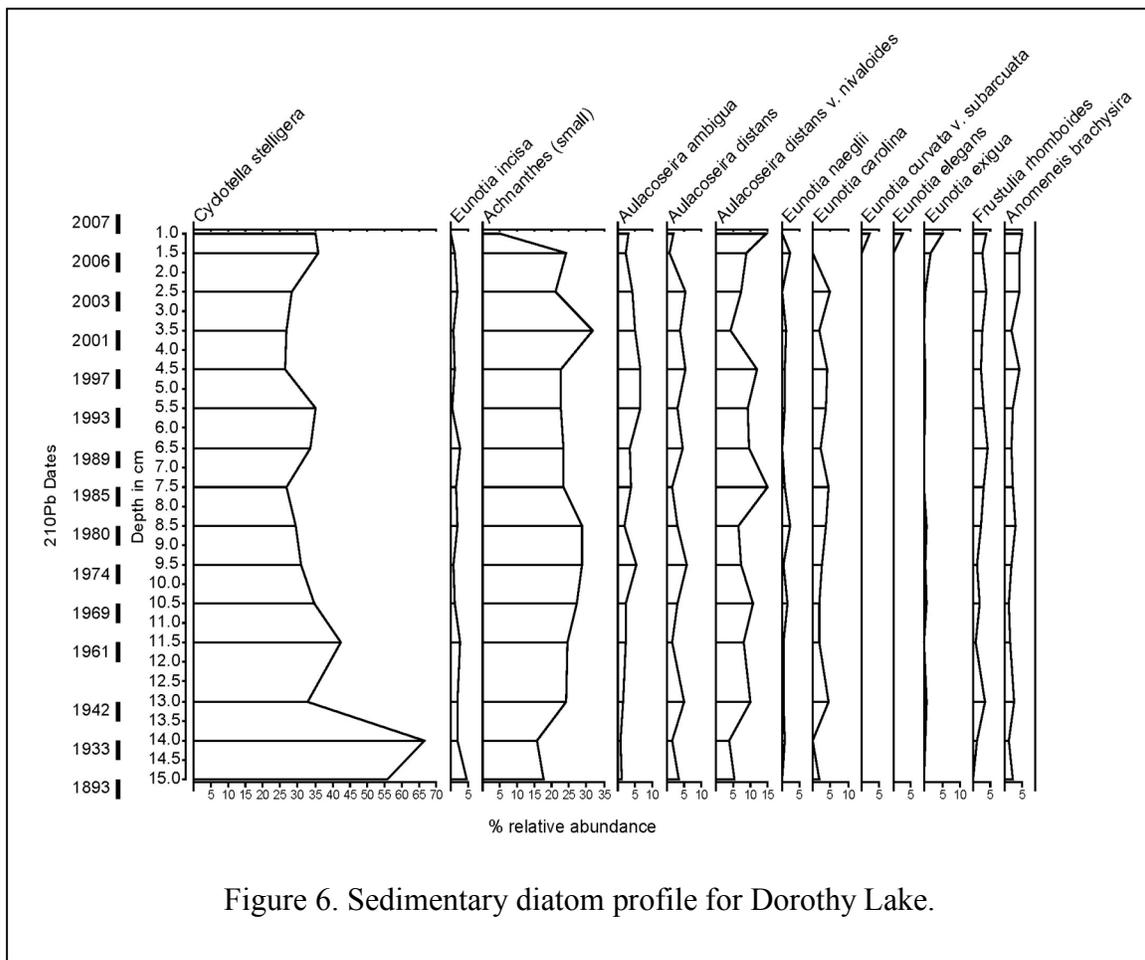
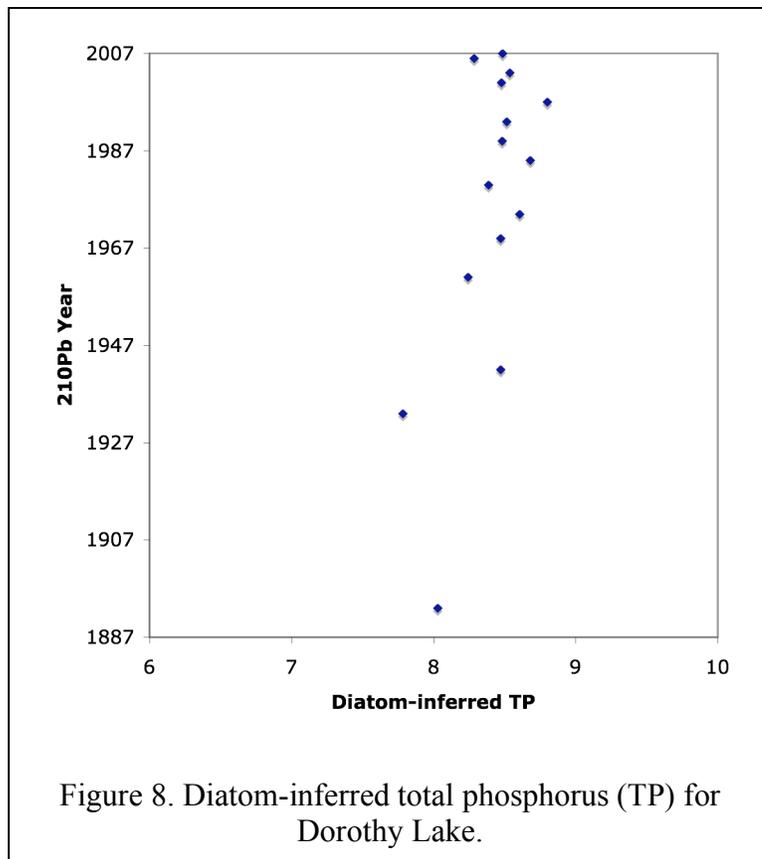
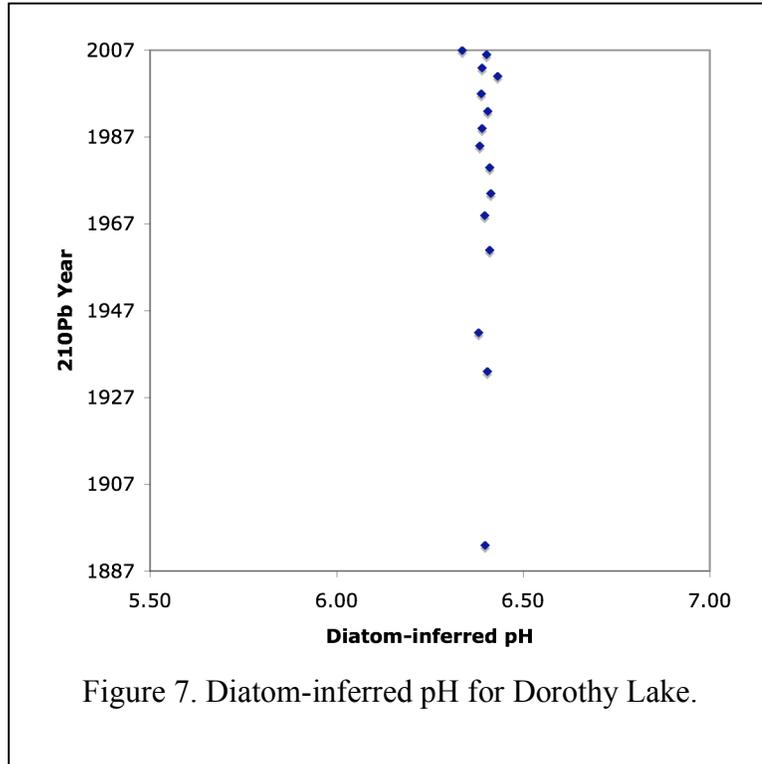


Figure 6. Sedimentary diatom profile for Dorothy Lake.



## CONCLUSIONS

Overall, the results of these experiments and lake surveys reveal that both lakes currently have sufficient N for algal growth, and are P-limited lakes. The paleolimnological results reveal that, while some changes in the relative abundances of diatom taxa have occurred during the last century, these changes do not suggest that any major shifts in pH or total phosphorus have occurred during that time.

These results suggest that N deposition has not had detectable effects on the diatom communities in these lakes. The current water chemistry further suggests that N deposition will not have enrichment effects on these lakes.

As the seston ratios provided accurate assessments of initial nutrient limitation patterns in this study, and this parameter is relatively easy to measure, a broader assessment of the potential sensitivity of lakes in the Northwest to N deposition would be possible via a survey of these seston parameters. Such research should also be carried out at higher elevation sites, as alpine lakes may exhibit different responses to N deposition than the subalpine systems investigated here.

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